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Research Article

Three new species of *Colletotrichum* (Glomerellales, Glomerellaceae) associated with walnut (*Juglans regia*) anthracnose from China

Yixuan Li[1,2], Lu Lin[1,2], Jing Cao[3], Mingxu Gan[4], Xinlei Fan[1,2]

1 *State Key Laboratory of Efficient Production of Forest Resources, Beijing Forestry University, Beijing 100083, China*
2 *Key Laboratory for Silviculture and Conservation of the Ministry of Education, Beijing Forestry University, Beijing 100083, China*
3 *Ankang Forestry Technology Promotion Centre, Ankang, Shaanxi 725099, China*
4 *Shangluo Forestry Extension Centre, Shangluo, Shaanxi 726199, China*

Corresponding author: Xinlei Fan (xinleifan@bjfu.edu.cn)

Abstract

Colletotrichum species are significant pathogens of various economic plant hosts worldwide. In this study, 45 *Colletotrichum* isolates were obtained from symptomatic walnut leaves of walnut anthracnose in Shaanxi and Sichuan Provinces. In conjunction with morphological evidence and multi-gene phylogenetic analyses of internal transcribed spacer (ITS), actin (*act*), chitin synthase 1 (*chs1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and beta-tubulin (*tub2*) sequences support the introduction of three new species, namely *Colletotrichum cordae*, *C. guangyuanense* and *C. juglandium*. Five species of *Colletotrichum* were identified to be *C. fioriniae* of the *C. acutatum* species complex, *C. karsti* of the *C. boninense* species complex, *C. gloeosporioides*, *C. mengyinense* and *C. siamense* of the *C. gloeosporioides* species complex. The three new species are described and illustrated in this paper and compared with taxa in the *Colletotrichum gloeosporioides* species complex. The current results improve the understanding of *Colletotrichum* species causing walnut anthracnose in China.

Key words: Glomerellaceae, novel species, systematics, taxonomy

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Introduction

Walnut (*Juglans regia* L.) is an economically significant woody nut and edible oil tree cultivated globally. It is widely grown across various regions in America, Asia, and Europe (Liu et al. 2021). According to the FAO statistics (http://www.fao.org/faostat, accessed on 20 March 2024), China is recognized as the world's largest walnut producer, with over 390,000 hectares dedicated to walnut cultivation (Liu et al. 2021). Since 2017, China has consistently maintained its leading position in global walnut production (Da Lio et al. 2018; Li et al. 2023). Walnut plantations offer substantial economic, social, and ecological benefits (Nie et al. 2016). However, anthracnose induced by *Colletotrichum* species remains a major hurdle in walnut production worldwide, causing significant losses in productivity, including total crop failures (Wang et al. 2016). For example, *Colletotrichum aenigma*, *C. fructicola*, *C. gloeosporioides*, *C. liaoningense*, *C. siamense* and *C. sojae* were reported to cause anthracnose of walnut in Beijing Province (Li et al. 2023).

Colletotrichum (Glomerellaceae, Glomerellales, Sordariomycetes) is one of the most important and destructive plant pathogens worldwide (Dean et al. 2012). Traditionally, identifying *Colletotrichum* species based solely on morphological characteristics and host ranges has been challenging (Walker 1980). Consequently, systematic studies of *Colletotrichum* species complexes have underscored the importance of a multiphasic approach, integrating locus phylogeny with morphological, geographical, and ecological data to accurately characterize and identify *Colletotrichum* species (Cai et al. 2009; Damm et al. 2009, 2012a, b; Rojas et al. 2010; Liu et al. 2011; Weir et al. 2012; Marin-Felix et al. 2017; Jayawardena et al. 2020). The current taxonomy of the genus encompasses over 300 species, organized into 16 species complexes, with additional singletons (Marin-Felix et al. 2017; Liu et al. 2022; Talhinhas and Baroncelli 2023; Zapata et al. 2024).

Research on walnut anthracnose in China's primary walnut-producing regions has identified fourteen *Colletotrichum* species associated with the disease (Zhang et al. 2023a). *Colletotrichum fructicola*, *C. gloeosporioides*, *C. siamense* and *C. viniferum* have been reported to be associated with walnut anthracnose in Shandong Province (Zhu et al. 2014; Wang et al. 2017, 2018; He et al. 2019). Moreover, *C. aenigma* has been implicated in Hebei Province, *C. fioriniae* in Guangxi Province, and *C. nymphaeae* in Gansu Province as pathogens of walnut anthracnose (Zhu et al. 2015; Wang et al. 2021; Ma et al. 2022). In Hubei Province, additional species including *C. fioriniae*, *C. gloeosporioides*, *C. godetiae*, *C. juglandis*, *C. kahawae*, and *C. nymphaeae* have been reported in association with the disease (Wei et al. 2022). *Colletotrichum godetiae* has been identified as a cause of severe anthracnose in walnuts in Shaanxi and Yunnan Provinces. Additionally, in Beijing, a range of species including *C. aenigma*, *C. fructicola*, *C. gloeosporioides*, *C. juglandicola*, *C. liaoningense*, *C. peakense*, *C. siamense*, and *C. sojae* have been reported (Li et al. 2023; Wang et al. 2023; Zhang et al. 2023a). Based on previous studies of walnut anthracnose, it is generally accepted that *C. gloeosporioides* is the main pathogen of walnut anthracnose in China (Qu et al. 2011; Wang et al. 2016). Furthermore, recent studies have revealed a diverse array of *Colletotrichum* species associated with walnut anthracnose, such as *C. acutatum*, *C. aenigma*, *C. fioriniae*, *C. fructicola*, and *C. siamense*, among others, as noted by Li et al. (2023). Therefore, the exploration of pathogen diversity within walnut anthracnose continues to be an essential field of study. In this study we investigated the phylogenetic diversity of *Colletotrichum* species associated with walnut anthracnose in Shaanxi and Sichuan Provinces. We aimed to classify the isolates from this study based on phylogenetic analyses and morphological characteristics.

Materials and methods

Sample Collection and fungal isolation

A total of 45 isolates were isolated from 25 walnut leaf samples with symptoms of anthracnose and collected in Shaanxi and Sichuan Provinces in China. The walnut anthracnose is characterized by small brown or black dry spots (Fig. 1).

Figure 1. Disease symptoms on *Juglans regia* L. caused by *Colletotrichum* species (**A–F**). Red arrows point to symptoms of branches dieback caused by walnut anthracnose.

About 25 mm^2 tissue fragments were taken from the margin of tissue lesions and the surfaces were sterilized with 75% ethanol for 30 s and 5% sodium hypochlorite for 60 s, rinsed in sterile distilled water for 60 s, and the samples were dried on aseptic filter paper (Gao et al. 2013; Liu et al. 2015). The sterilized sample was then placed in potato dextrose agar (PDA, 200 g potato, 20 g glucose, 20 g agar and 1 L distilled water) and cultured at 25 °C until mycelium grew from the sample. Then hyphae were picked out of the periphery of the colonies and inoculated onto Oatmeal Agar (OA, 30 g oatmeal, 15 g agar and 1 L distilled water) medium to promote the formation of spores. These leaf specimens are kept at the Museum of Beijing Forestry University (BJFC). The cultures are deposited in the China Forestry Culture Collection Centre (CFCC; http://www.cfcc-caf.org.cn/).

Morphological analyses

Conidial structure and size were imaged with Leica stereo microscope (M205) (Leica Microsystems, Wetzlar, Germany). Conidia and other microstructures were randomly selected and observed by using Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan) equipped with a Nikon digital sight DSRi-2 high-definition color camera with differential interference contrast (DIC). Fifty conidia were selected randomly to measure their lengths and widths. Colony morphology was observed on PDA and OA cultured at 25 °C. According to the color map of Rayner (1970) the color of the colony was described. The colony diameter was measured after 5 and 14 days.

DNA extraction, PCR amplification and sequencing

Mycelium was collected from isolates grown on PDA agar and genomic DNA extraction was performed using the modified CTAB method (Doyle and Doyle 1990). First, the internal transcribed spacer (ITS) of all isolates was sequenced. The other genes were obtained from five nuclear gene regions: the glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*), chitin synthase 1 gene (*chs1*), actin gene (*act*), beta-tubulin gene (*tub2*) and histone H3 gene (*his3*) by using the primer pairs GDF1/GDR1, CHS-79F/CHS-345R, ACT-512F/ACT-783R, T1/Bt2b and CYLH3F/CYLH3R, respectively. The total volume of the PCR mixture is 20 µL, including 1 µL DNA template, 1 µL each 10 µM primer, 10 µL T5 Super PCR Mix and 7 µL sterile water. The gene fragments and amplification conditions used were in accordance with the details shown in Table 1 (Liu et al. 2022). The PCR products were electrophoresed in 1% agarose gel, and the DNA was sequenced by Sino Geno Max Biotechnology Company Limited (Beijing, China).

Phylogenetic analyses

The resulting DNA sequences were combined with the sequences of reference strains from Genbank (Supplementary Suppl. material 1), and each single-gene dataset was aligned on MAFFT v. 6 separately (Katoh and Standley 2013), with both ends cut. Phylogenetic analysis of *C. acutatum* and *C. boninense* species complex was performed by combining six loci (ITS, *act*, *chs1*, *gapdh*, *his3* and *tub2*). Phylogenetic analysis of *C. gloeosporioides* species complex was performed by combining five loci (ITS, *act*, *chs1*, *gapdh*, and *tub2*). *Colletotrichum bambusicola* LC8469 *and C. orchidophilum* CBS 632.80 were used as the outgroup. Phylogenetic analyses of Maximum Likelihood (ML) and Bayesian Inference (BI) were performed. ML and BI analyses were computed using PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). For BI analysis, the best-fitting evolutionary model for each partitioned locus was estimated using the Markov Chain Monte Carlo algorithm in MrModelTest v. 2.3 (Posada and Crandall 1998). The system diagram is plotted in FigTree v. 1.4.3 (Rambaut and Drummond 2010) (http://tree.bio.ed.ac.uk/software/figtree) and edited in Adobe Illustrator 2019 (https://www.adobe.com/cn/products/illustrator.html). Sequence data were submitted to GenBank (https://www.ncbi.nlm.nih.gov) (Suppl. material 1).

Table 1. Genes used in this study with PCR primers and optimal annealing temperature.

Locus	PCR Primers	PCR: Thermal Cycles: (Annealing Temp. in Bold)	Reference
ITS	ITS1/ITS4	(95 °C: 30 s, 51 °C: 30 s, 72 °C: 1 min) × 35 cycles	White et al. (1990)
act	ACT-512F/ACT-783R	(95 °C: 45 s, 55 °C: 45 s, 72 °C: 1 min) × 35 cycles	Carbone and Kohn (1999)
chs1	CHS-79F/CHS-345R	(95 °C: 30 s, 58 °C: 30 s, 72 °C: 1 min) × 35 cycles	Carbone and Kohn (1999)
gapdh	GDR1/GDF1	(95 °C: 30 s, 58 °C: 30 s, 72 °C: 1 min) × 35 cycles	Guerber et al. (2003)
his3	CYLH3F/CYLH3R	(95 °C: 30 s, 58 °C: 30 s, 72 °C: 1 min) × 35 cycles	Crous et al. (2004)
tub2	T1/Bt2b	(95 °C: 30 s, 55 °C: 30 s, 72 °C: 1 min) × 35 cycles	Glass and Donaldson (1995)

Results

Phylogenetic analyses

Forty-five strains of *Colletotrichum*, isolated from leaves of *Juglans regia* L., were identified based on phylogenetic analyses of six loci. In the phylogenetic analysis of the *C. acutatum* species complex, a total of 2238 characters, including gaps, were identified (ITS: 549, *act*: 248, *chs1*: 282, *gapdh*: 267, *his3*: 390 and *tub2*: 502). Similarly, the phylogenetic analysis of the *C. boninense* species complex yielded a total of 2639 characters, including gaps (ITS: 592, *act*: 248, *chs1*: 300, *gapdh*: 321, *his3*: 410 and *tub2*: 768). An additional analysis of the *C. gloeosporioides* species complex resulted in 2294 characters, including gaps (ITS: 575, *act*: 323, *chs1*: 300, *gapdh*: 348 and *tub2*: 748). The GTR+I+G model was proposed for ITS, *act* and *gapdh*, and the HKY+I+G model was proposed for *chs1*, *his3* and *tub2* (Ronquist and Huelsenbeck 2003). The best-fit models used the statistics of ML trees are shown in Suppl. material 2. Both Maximum Likelihood (ML) and Bayesian Inference (BI) methods were employed in these analyses. The topology of Bayesian analysis of cascading datasets is almost the same as ML consistency tree.

The phylogenetic tree showed 45 isolates across three species complexes: the *C. acutatum* species complex with a single isolate (Fig. 2), the *C. boninense* species complex with a single isolate (Fig. 3), and the *C. gloeosporioides* species complex with 43 isolates (Fig. 4). In the *C. acutatum* species complex, one isolate clustered with four reference isolates of *C. fioriniae*. In the *C. boninense* species complex, one isolate clustered with seven reference isolates of *C. karsti*. In the *C. gloeosporioides* species complex, 14 isolates clustered together with *C. mengyinense*, 17 isolates clustered with *C. gloeosporioides* and four isolates clustered with *C.siamense*, eight isolates formed three separate clades with high support (Fig. 4).

Taxonomy

***Colletotrichum cordae* Y.X. Li & X.L. Fan, sp. nov.**

MycoBank No: 852127

Fig. 5

Etymology. Named after Corda who established the genus *Colletotrichum*.

Typification. CHINA, Sichuan Province, Guangyuan City, Chaotian District, Longmen Valley Leisure Villa, 32°39'08"N, 105°55'11"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (holotype BJFC-S2250, ex-holotype culture CFCC 59618).

Description. ***Sexual morph*** not observed. ***Asexual morph*** developed on OA. ***Conidiomata*** acervular, color ranged from peach to light brown. ***Appressoria*** and ***Setae*** not observed on OA. ***Conidiophores*** directly formed on hyphae, usually degenerated into conidiogenous cells. ***Conidiophores*** hyaline, unbranched, approximately cylindrical, 16.1–28.2 × 2.5–4.5, mean ± SD = 21.0 ± 2.9 × 3.5 ± 0.5 µm, n = 50. ***Conidiogenous cells*** transparent, cylindrical, formed at the end or side of the hyphae. ***Conidia*** straight, hyaline, cylindrical, obtuse at the base, rounded at the apex, with smooth walls and granular contents, 11.8–17.7 × 4.5–6.5 µm, mean ± SD = 14.5 ± 1.1 × 5.5 ± 0.5 µm, L/W radio = 2.6, n = 50.

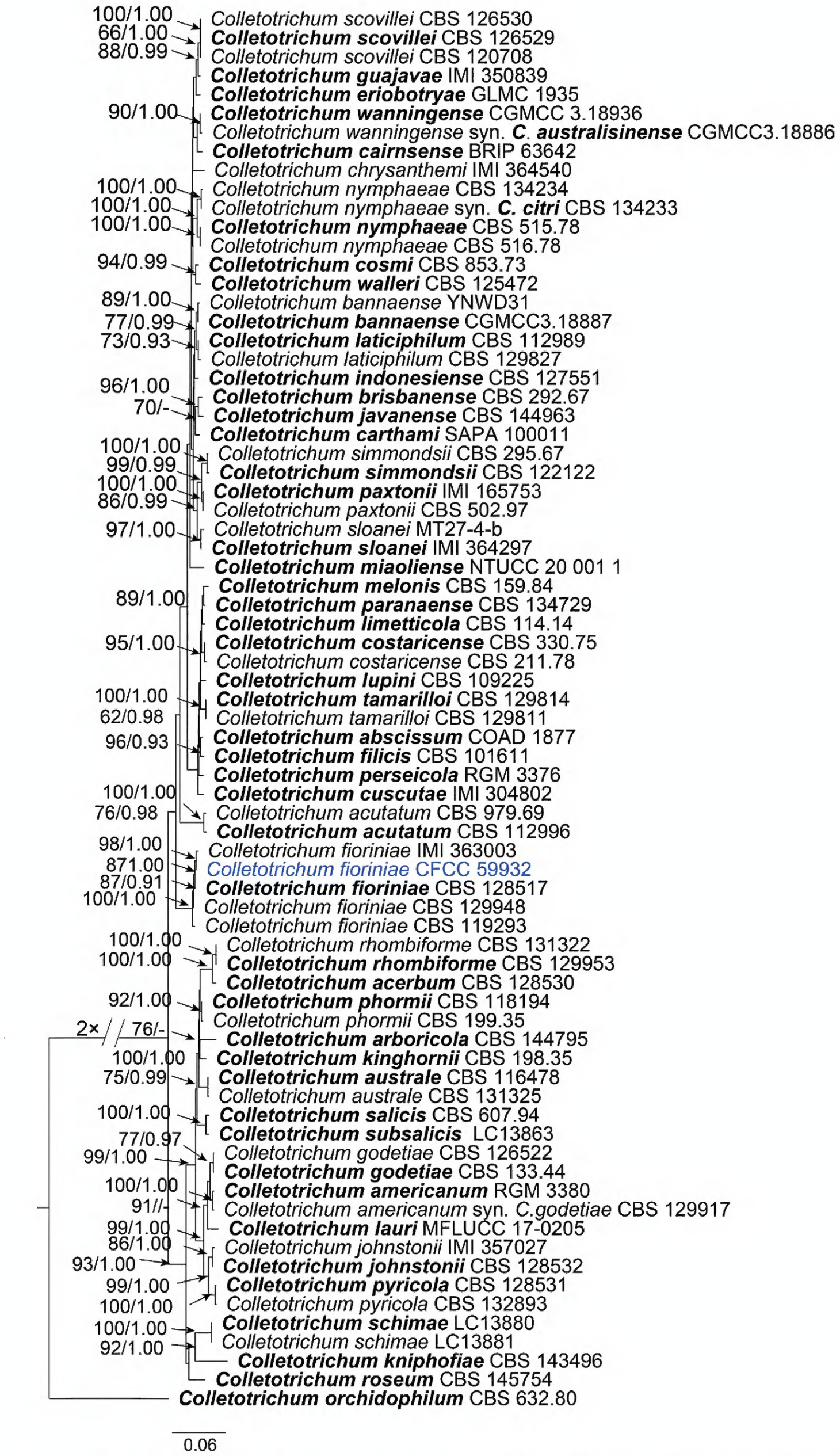

Figure 2. Phylogram of *Colletotrichum acutatum* complex species based on Maximum Likelihood (ML) analysis of the dataset of combined ITS, *gapdh*, *act*, *tub2*, *chs1* and *his3* genes. ML bootstrap support values above 60% and Bayesian posterior probability above 0.90 are shown near nodes. Ex-type cultures are in bold. Isolates obtained in this study are highlighted with blue colors.

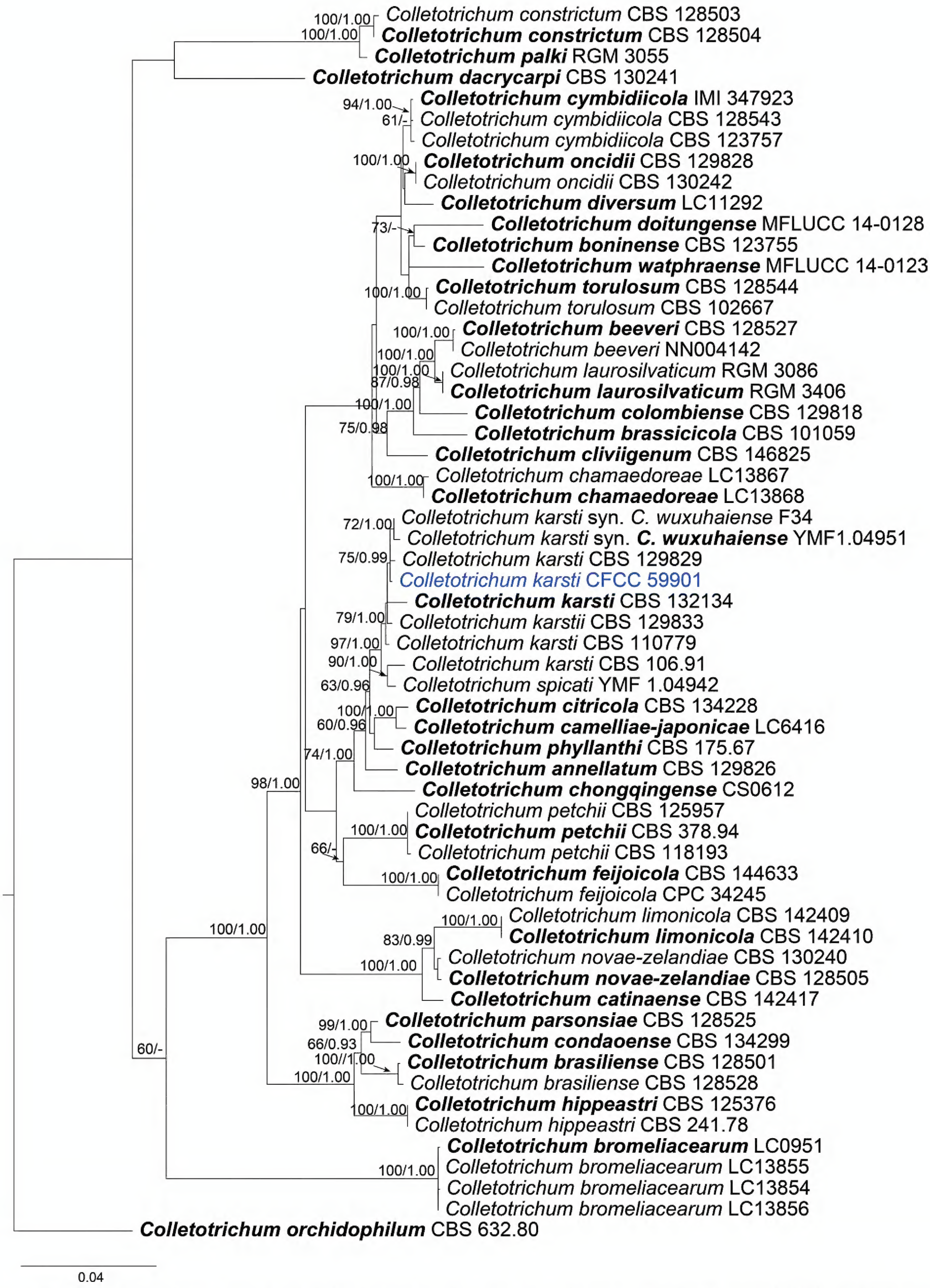

Figure 3. Phylogram of *Colletotrichum boninense* complex species based on Maximum Likelihood (ML) analysis of the dataset of combined ITS, *gapdh*, *act*, *tub2*, *chs1* and *his3* genes. ML bootstrap support values above 60% and Bayesian posterior probability above 0.90 are shown near nodes. Ex-type cultures are in bold. Isolates obtained in this study are highlighted with blue colors.

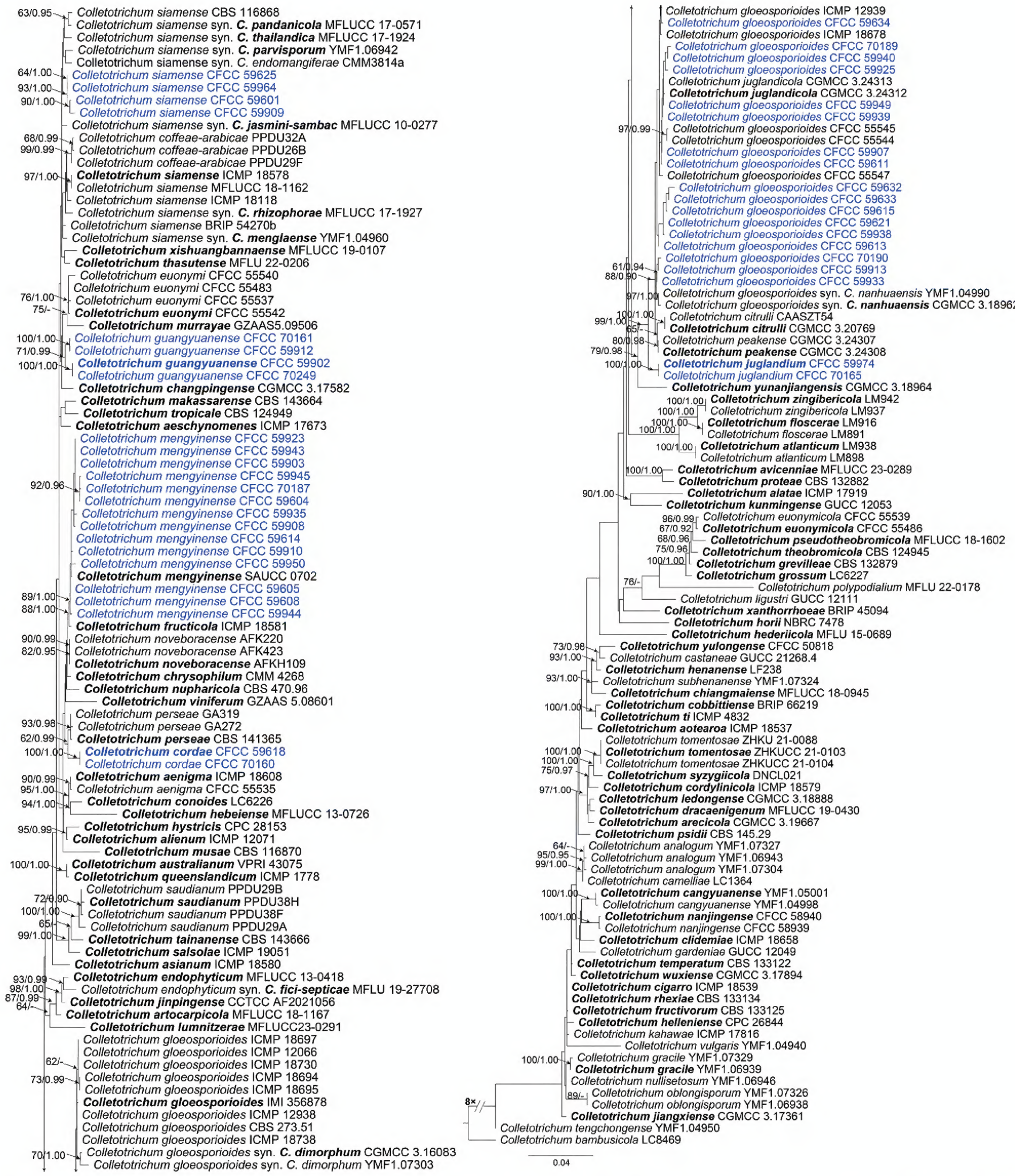

Figure 4. Phylogram of *Colletotrichum gloeosporioides* complex species based on Maximum Likelihood (ML) analysis of the dataset of combined ITS, *gapdh*, *act*, *tub2* and *chs1* genes. ML bootstrap support values above 60% and Bayesian posterior probability above 0.90 are shown near nodes. Ex-type cultures are in bold. Isolates obtained in this study are highlighted with blue colors.

Cultural characteristics. Colonies on OA initially white, rapidly growing to 4 cm after 3 d at 25 °C, and completely covering a 6-centimeter Petri dish after 7 d. The aerial mycelium white or gray, with a flocculent cotton-like appearance, edge white, center iron gray.

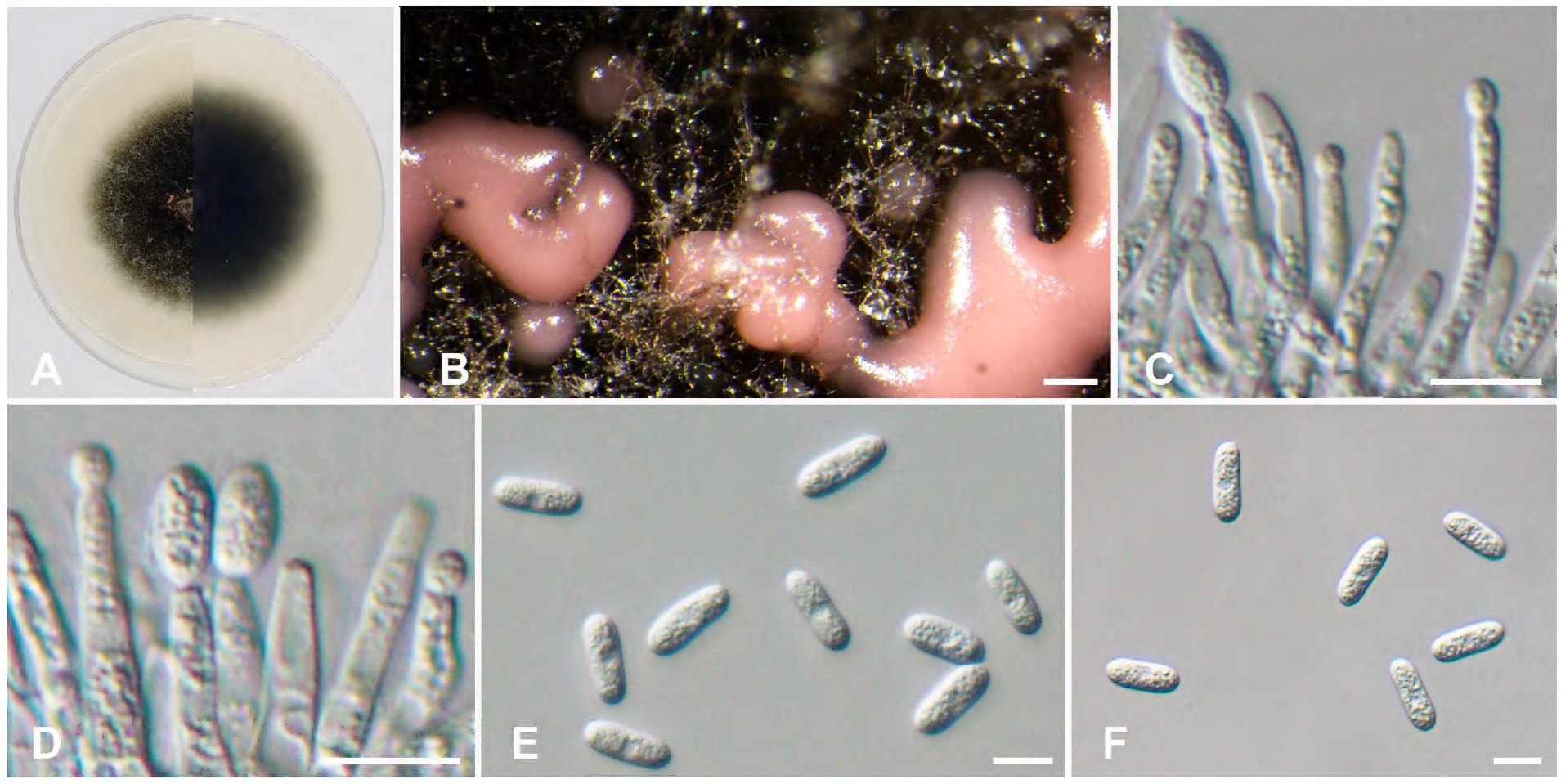

Figure 5. *Colletotrichum cordae* (ex-holotype culture CFCC 59618) **A** colonies on OA media above and below after 5 days at 25 °C **B** conidiomata on OA **C, D** conidiophores and conidia **E, F** conidia. Scale bars: 200 μm (**B**); 10 μm (**C–F**).

Additional material examined. CHINA. Sichuan Province, Guangyuan City, Chaotian District, Longmen Valley Leisure Villa, 32°39'08"N, 105°55'11"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2251, living culture CFCC 70160).

Notes. Two strains of *Colletotrichum cordae* constitute a distinct clade within the *C. gloeosporioides* species complex, as revealed by multi-locus phylogenetic analysis. *Colletotrichum cordae* is phylogenetically near to *C. perseae* CBS 141365, but differs by 17 nucleotide differences in concatenated alignment (7/573 in ITS, 5/281 in *act*, 1/269 in *chs1*, 1/317 in *gapdh*, and 3/452 in *tub2*) (Sharma et al. 2017). Morphologically, *C. cordae* can be differentiated from *C. perseae* by the presence of conidia with obtuse or rounded apices.

Colletotrichum fioriniae **(Marcelino & Gouli) R.G. Shivas & Y.P. Tan, Fungal Divers. 39: 117, 2009**

Material examined. CHINA. Shaanxi Province, Shangluo City, Lonan County, Red kernel walnut base, 34°03'10"N, 110°14'11"E, from leaf of *Juglans regia* L., 14 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2253, living culture CFCC 59932).

Notes. *Colletotrichum fioriniae*, a worldwide fungus with a wide range of host, is associated with walnut anthracnose disease (Shivas and Tan 2009; Zhu et al. 2015). Relative to other species within the *Colletotrichum* genus, *C. fioriniae* is generally considered less prevalent and less virulent, as noted by Talhinhas and Baroncelli (2021). In our research, the strain CFCC 59932 clusters robustly with *C. fioriniae* on both Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic trees, indicating strong statistical support (ML/BI = 100/1).

***Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., Atti Reale Ist. Veneto Sci. Lett. Arti., ser. 6, 2: 670. 1884**

Materials examined. CHINA. • Sichuan Province, Guangyuan City, Chaotian District, Walnut Cultural Square, 32°40'58"N, 106°02'08"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2239, living culture CFCC 59613; BJFC-S2240, living culture CFCC 59621; BJFC-S2242, living culture CFCC 59634); • Chaotian District, Mianguang Expressway, 32°40'50"N, 105°59'19"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2246, living culture CFCC 59611; BJFC-S2247, living culture CFCC 59615; BJFC-S2244, living culture CFCC 59632); • Chaotian District, Zhongzi Town, 32°41'34"N, 106°02'23"E, from leaf of *Juglans regia* L, 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2230, living culture CFCC 59633; BJFC-S2228, living culture CFCC 59907; BJFC-S2252, living culture CFCC 59913; BJFC-S2254, living culture CFCC 59933). CHINA. • Shaanxi Province, Shangluo City, Danfeng County, Dihua Ancient Town, 33°44'23"N, 110°12'07"E, from leaf of *Juglans regia* L., 13 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2257, living culture CFCC 59938; BJFC-S2258, living culture 59939); • Danfeng County, Walnut Theme Park, 33°44'33"N, 110°11'55"E, from leaf of *Juglans regia* L., 13 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2263, living culture CFCC 59925; BJFC-S2264, living culture CFCC 70189; BJFC-S2259, living culture CFCC 59940; BJFC-S2267, living culture CFCC 59949; BJFC-S2261, living culture CFCC 70190).

Notes. *Colletotrichum gloeosporioides* was originally described as *Vermicularia gloeosporioides* and collected from *Citrus* sp. in Italy. The current name *Colletotrichum gloeosporioides* was proposed by Penzig (1882). *Colletotrichum gloeosporioides* is a worldwide fungus that inhabits a wide range of host plants. In our study, 17 strains and three species (i.e., *C. citrulli*, *C. juglandicola* and *C. peakense*) were robustly grouped with *C. gloeosporioides*, supported by high Maximum Likelihood (ML) and Bayesian Inference (BI) confidence values (ML/BI = 88/0.90). Zhang et al. (2023b) reduced *C. dimorphum* and *C. nanhuaense* as synonyms of *C. gloeosporioides*. Further research is needed to confirm the taxonomic status of *C. citrulli*, *C. juglandicola* and *C. peakense*. The morphology of the strains in our study closely resembles the type specimen of *C. gloeosporioides*, as described by Cannon et al. (2008). Thus, we propose the identification of our strains as *C. gloeosporioides*, based on both morphological characteristics and phylogenetic analyses. The result proves that walnut anthracnose has been attributed to *C. gloeosporioides* (Wang et al. 2020a; Mu et al. 2021; Yang et al. 2021; Li et al. 2023).

***Colletotrichum guangyuanense* Y.X. Li & X.L. Fan, sp. nov.**

MycoBank No: 852126

Fig. 6

Etymology. Named after the location where the fungal was first collected, which is Guangyuan City.

Typification. CHINA, Sichuan Province, Guangyuan City, Lizhou District, Shuiwo Village, 32°23'55"N, 105°39'24"E, from leaf of *Juglans regia* L., 11 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (holotype BJFC-S2225, ex-holotype culture CFCC 59902).

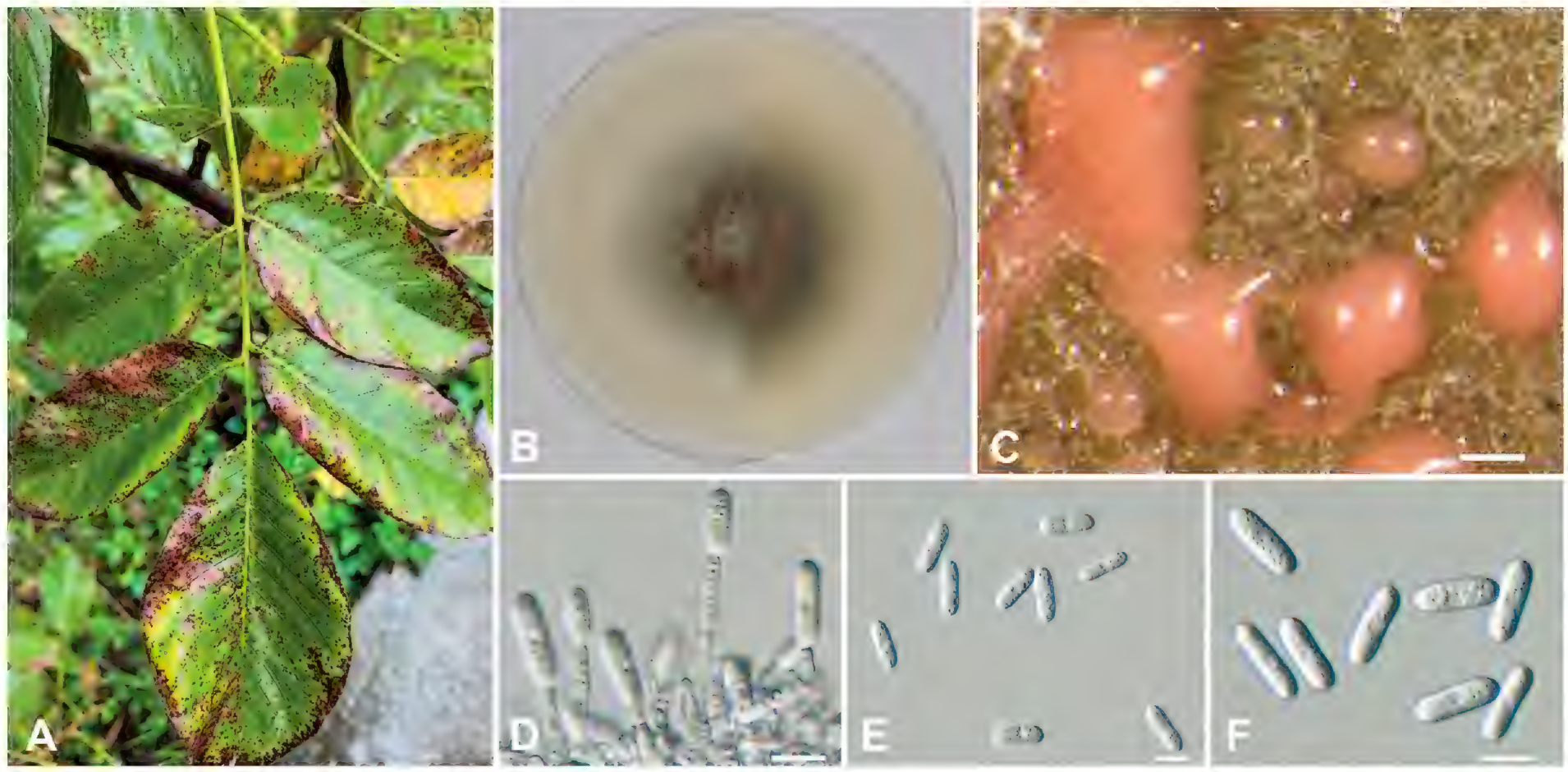

Figure 6. *Colletotrichum guangyuanense* (ex-holotype culture CFCC 59902) **A** symptom caused by *Colletotrichum guangyuanense* **B** colonies on OA media above and below after 5 days at 25 °C **C** conidiomata on OA **D** conidiophores and conidia **E, F** conidia. Scale bars: 200 μm (**C**); 10 μm (**D–F**).

Description. ***Sexual morph*** not observed. ***Asexual morph*** developed on OA. ***Conidiomata*** acervular, color ranged from peach to light brown. ***Appressoria*** and ***Setae*** not observed on OA. ***Conidiophores*** hyaline, unbranched, approximately cylindrical, 22.2–35.1 × 3.2–5.3, mean ± SD = 27.4 ± 3.1 × 3.8 ± 0.5 μm, n = 50. ***Conidiogenous cells*** transparent, cylindrical, formed at the end or side of the hyphae. ***Conidia*** straight, hyaline, cylindrical, obtuse at the base, rounded at the apex, with smooth walls and granular contents, 9.7–17.7 × 3.9–6.9 μm, mean ± SD = 14.7 ± 1.9 × 5.5 ± 0.6 μm, L/W radio = 2.7, n = 100.

Culture characteristics. Colonies on OA initially white, rapidly growing to 5 cm after 3 d at 25 °C, and completely covering a 6 cm Petri dish after 7 d. The aerial mycelium white or gray, with a flocculent cotton like appearance, edge white, center pale greenish grey.

Additional materials examined. China. • Sichuan Province, Guangyuan City, Liz-hou District, Shuiwo Village, 32°23'31"N, 105°39'22"E, from leaf of *Juglans regia* L., 11 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2226, living culture CFCC 70249). China. • Sichuan Province, Guangyuan City, Chaotian District, Longmen Valley Leisure Villa, 32°39'08"N, 105°58'17"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2248, living culture CFCC 59912); • Chaotian District, Longmen Valley Leisure Villa, 32°39'11"N, 105°55'26"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2249, living culture CFCC 70161).

Notes. In phylogenetic analyses, *Colletotrichum guangyuanense* forms a distinct clade within the *C. gloeosporioides* species complex, closely related to *C. changpingense*. Genetic differences between *C. guangyuanense* and the type strain of *C. changpingense* are observed at several loci: 4 bp in the ITS region, 3 bp in the *act* gene, 8 bp in the *chs1* gene, 6 bp in the *gapdh* gene, and 1 bp in the *tub2* gene (Jayawardena et al. 2016a). Morphologically, *C. guangyuanense* is distinguishable from *C. changpingense* by the absence of a distinct opaque region in the center of the conidia.

Colletotrichum juglandium **Y.X. Li & X.L. Fan, sp. nov.**

MycoBank No: 852125

Fig. 7

Etymology. Named after the host genus on which it was collected, *Juglans regia* L.

Typification. CHINA. Sichuan Province, Guangyuan City, Chaotian District, Mianguang Expressway, 32°40'50"N, 105°59'19"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (holotype BJFC-S2243, ex-holotype culture CFCC 59974).

Description. ***Sexual morph*** not observed. ***Asexual morph*** on OA. ***Conidiomata*** acervular, color ranged from peach to light brown. ***Appressoria*** and ***Setae*** not observed on OA. ***Conidiophores*** hyaline, unbranched, approximately cylindrical, 16.0–27.6 × 2.2–4.7, mean ± SD = 20.1 ± 3.2 × 3.2 ± 0.6 µm, n = 30. ***Conidiogenous cells*** transparent, cylindrical, formed at the end or side of the hyphae. ***Conidia*** straight, hyaline, cylindrical, obtuse at the base, rounded at the apex, with smooth walls and granular contents, 13.2–22.4 × 4.4–6.3 µm, mean ± SD = 16.8 ± 1.8 × 5.4 ± 0.4 µm, L/W radio = 3.1, n = 50.

Culture characteristics. Colonies on OA initially white, rapidly growing to 5 cm after 3 d at 25 °C, and completely covering a Petri dish after 7 days. The aerial mycelium white or gray, with a flocculent cotton like, edge white, center mouse grey.

Additional material examined. CHINA. Sichuan Province, Guangyuan City, Chaotian District, Mianguang Expressway, 32°40'36"N, 105°59'26"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2245, living culture CFCC 70165).

Notes. *Colletotrichum juglandium* form a solitary clade the *C. gloeosporioides* species complex (Fig. 3). *Colletotrichum juglandium* is closely related to *C. citrulli*, *C. gloeosporioides*, *C. juglandicola* and *C. peakense* (Zhang et al. 2023a). Sequence identity comparisons reveal that *C. juglandium* CFCC 59974 differs from other species at various loci: *C. citrulli* CGMCC3.20769 (3/544 in ITS, 0/244 in *act*, 0/228 in *chs1*, 10/297 in *gapdh*, and 0/324 in *tub2*), *C. gloeosporioides* IMI 356878 (2/544 in ITS, 0/289 in *act*, 0/236 in *chs1*, 18/341 in *gapdh*, and 0/324 in *tub2*), *C. juglandicola* CGMCC 3.24312 (2/544 in ITS, 0/279 in *act*, 1/249 in *chs1*, 18/341 in *gapdh*, and 1/324 in *tub2*), and *C. peakense* CGMCC 3.24308 (2/544 in ITS, 0/279 in *act*, 1/249 in *chs1*, 13/341 in *gapdh*, and 1/324 in *tub2*) (Guo et al. 2022; Yu et al. 2022; Zhang et al. 2023a). Morphologically, *C. juglandium* differs from *C. gloeosporioides* by having narrower conidia (L/W ratio: 3.1 vs. 2.6) and differs from *C. juglandicola* and *C. peakense* by having wider conidia (L/W ratio: 3.1 vs. 3.3).

Colletotrichum karsti **Y.L. Yang, Z.Y. Liu, K.D. Hyde & L. Cai, Cryptog. Mycol. 32: 241. 2011**

Material examined. CHINA. Sichuan Province, Guangyuan City, Lizhou District, Shuiwo Village, 32°23'38"N, 105°39'22"E, from leaf of *Juglans regia* L., 11 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2224, living culture CFCC 59901).

Notes. *Colletotrichum karsti* was identified as a pathogen on *Vanda* species, causing ellipsoid lesions on leaves, and was also found as an endophyte in

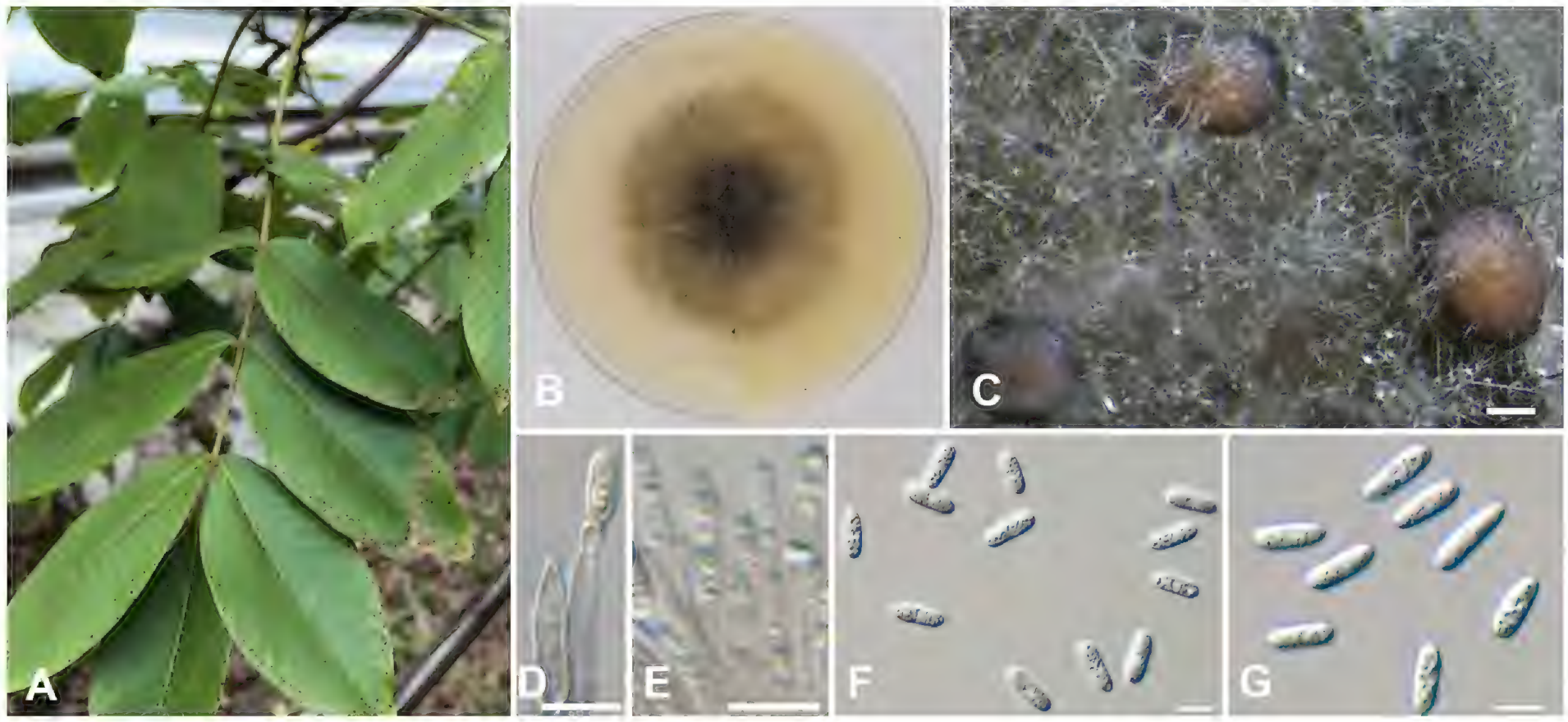

Figure 7. *Colletotrichum juglandium* (ex-holotype culture CFCC 59974) **A** symptoms caused by *Colletotrichum juglandium* **B** colonies on OA media above and below after 5 days at 25 °C **C** conidiomata **D, E** conidiophores and conidia **F, G** conidia. Scale bars: 200 μm (**C**); 10 μm (**D–G**).

roots in Guizhou Province, China. This species is recognized as the most prevalent and geographically widespread within the *C. boninense* species complex, with a broad host range (Yang et al. 2011; Damm et al. 2012a; Jayawardena et al. 2016b). Zhang et al. (2022) synonymized *C. wuxuhaiense* with C. *karsti*. In this study, the strain CFCC 59901 is confirmed to be *C. karsti* based on morphological characteristics and DNA sequence data. Furthermore, this represents a new host record for *C. karsti* on walnut trees.

Colletotrichum mengyinense **T.C. Mu, J.W. Xia, X.G. Zhang & Z. Li, MycoKeys 85: 66 (2021)**

Materials examined. CHINA. • Sichuan Province, Guangyuan City, Chaotian District, Walnut Cultural Square, 32°40'58"N, 106°02'08"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2241, living culture CFCC 59604; BJFC-S2236, living culture CFCC 59605; BJFC-S2237, living culture CFCC 59608; BJFC-S2238, living culture CFCC 59910); • Chaotian District, Cypress Bridge, 32°41'16"N, 106°02'22"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2232, living culture CFCC 59614); • Chaotian District, Zhongzi Town, 32°41'34"N, 106°02'23"E, from leaf of *Juglans regia* L., 10 Oct. 2023,Y.X. Li, L. Lin & X.L. Fan (BJFC-S2229, living culture CFCC 59908); • Lizhou District, Tulongzi, 32°30'36"N, 105°36'51"E, from leaf of *Juglans regia* L., 11 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2227, living culture CFCC 59903). CHINA. • Shaanxi Province, Shangluo City, Danfeng County, Walnut Theme Park, 33°44'33"N, 110°11'55"E, from leaf of *Juglans regia* L., 13 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2260, living culture CFCC 59923; BJFC-S2262, living culture CFCC 59943; BJFC-S2265, living culture CFCC 59944; BJFC-S2266, living culture CFCC 59945; BJFC-S2268, living culture CFCC 59950); • Shangzhou District, United Village, 33°52'03"N, 109°51'01"E, from leaf of *Juglans regia* L.,

14 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2256, living culture CFCC 59935; BJFC-S2255, living culture CFCC 70187).

Notes. *Colletotrichum mengyinense* was isolated originally on diseased leaves of *Rosa chinensis* (Mu et al. 2021). Additionally, the current 14 isolates are morphologically not significantly different from *C. mengyinense* and aggregated together with *C. mengyinense* with high support (ML/BI = 89/1.00) on the phylogenetic tree. Therefore, they are identified as *Colletotrichum mengyinense*.

***Colletotrichum siamense* Prihastuti, L. Cai & K.D. Hyde, Fungal Divers. 39: 98 (2009)**

Materials examined. CHINA. • Sichuan Province, Guangyuan City, Chaotian District, Zhongzi Town, 32°41'05"N, 106°02'08"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (BJFC-S2231, living culture CFCC 59601; BJFC-S2233, living culture CFCC 59625; BJFC-S2235, living culture CFCC 59964); • 32°41'34"N, 106°02'23"E, from leaf of *Juglans regia* L., 10 Oct. 2023, Y.X. Li, L. Lin & X.L. Fan (holotype BJFC-S2234, living culture CFCC 59909)

Notes. *Colletotrichum siamense* was first described as a species in association with *Coffea arabica* by Prihastuti et al. (2009). The broader concept of *C. siamense sensu lato* has been a subject of considerable debate, as noted by Weir et al. (2012) and Sharma et al. (2015), due to the application of the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) approach. Liu et al. (2016) concluded that *C. siamense* is a single species, not a species complex. Based on phylogenetic evidence, Zhang et al. (2022) proposed that *C. menglaense*, *C. pandanicola*, and *C. parvisporum* are synonyms of *C. siamense*. More recent studies have further synonymized the closely related species *C. rhizophorae* and *C. thailandica* with *C. siamense*, considering morphological characteristics, phylogenetic analyses, and GCPSR (Aumentado et al. 2024). In this study, *C. siamense* was isolated from walnut leaf spots affected by walnut anthracnose. The four isolates our study examined while forming a distinct lineage were found within the *C. siamense* clade in our phylogenetic analysis. Moreover, our isolate (CFCC 59909) is similar to the holotype of *C. siamense* (ICMP 18578). While the conidia of our isolate (CFCC 59909) are wider than strain ICMP 18578 (12.3–14.5 × 4.9–6.4 vs. 7–18.3 × 3–4.3 µm) (Prihastuti et al. 2009). Based on this evidence, we identify our isolate as *C. siamense*.

Discussion

The genus *Colletotrichum* comprises significant plant pathogens that impact many economically important crops globally. Despite notable advancements in the taxonomy of *Colletotrichum*, ongoing debates regarding its taxonomic relationships warrant further research (Cannon et al. 2008, 2012; Cai et al. 2009; Liu et al. 2013, 2016). This study focused on walnut anthracnose in Sichuan and Shaanxi Provinces of China, conducting phylogenetic analyses using DNA sequence data. Five known species (*C. fioriniae*, *C. gloeosporioides*, *C. karsti*, *C. mengyinense* and *C. siamense*) and three new species (*C. cordae*, *C. guangyuanense* and *C. juglandium*) associated with walnut anthracnose were identified in the current study.

The genus *Colletotrichum* exhibits considerable species diversity in infections of walnut hosts. In China, a variety of *Colletotrichum* species have been reported on walnut, including those from the *C. acutatum* species complex (e.g., *C. acutatum*, *C. fioriniae*, *C. godetiae*, *C. juglandicola*, *C. juglandis*, *C. nymphaeae*), the *C. gloeosporioides* species complex (e.g., *C. aenigma*, *C. fructicola*, *C. gloeosporioides*, *C. kahawae*, *C. mengyinense*, *C. peakense*, *C. siamense*, *C. viniferum*), the *C. magnum* species complex (e.g., *C. liaoningense*), and the *C. orchidearum* species complex (e.g., *C. sojae*). This list is based on numerous studies conducted over the years (Simmonds 1966; Alvarez 1976; Gorter 1977; Pennycook 1989; Liu et al. 1995; Chen 2003; Gadgil et al. 2005; Juhásová et al. 2005; Sreenivasaprasad and Talhinhas 2005; Kobayashi 2007; Qu et al. 2011; Damm et al. 2012b; Zhu et al. 2014, 2015; Wang et al. 2017, 2018; Da Lio et al. 2018; He et al. 2019; Savian et al. 2019; Wang et al. 2020b, 2021; Luongo et al. 2022; Ma et al. 2022; Wei et al. 2022; Li et al. 2023; Wang et al. 2023; Zhang et al. 2023a). Pathogenicity tests have demonstrated that numerous *Colletotrichum* species are responsible for anthracnose disease on walnut fruits and leaves, as Li et al. (2023) and Zhang et al. (2023a) reported. Wang et al. (2020a) revealed that the virulence of the pathogen of walnut anthracnose to walnut fruits was different. In this study, the pathogenicity of two established species, *C. mengyinense* and *C. karsti*, and three newly identified species, requires further investigation.

In the current study, five species (i.e., *C. citrulli*, *C. dimorphum*, *C. juglandicola*, *C. nanhuaense* and *C. peakense*) along with 17 isolates form part of the *C. gloeosporioides* clade in both Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic trees. However, these species and isolates were unable to form clear branches in the phylogenetic tree. They also have overlapping morphological characters (Guo et al. 2022; Yu et al. 2022; Zhang et al. 2023a). Consequently, the 17 isolates in question have been identified as *C. gloeosporioides*. Liu et al. (2022) demonstrated that the boundaries between *C. gloeosporioides* and its closely related species are unclear. Zhang et al. (2023b) synonymized *C. dimorphum* and *C. nanhuaense* with *C. gloeosporioides*. Thus, we propose that *C. citrulli*, *C. juglandicola*, and *C. peakense* be considered as synonyms of *C. gloeosporioides*. Further genome-wide data studies are needed to clarify the species boundaries in this large clade (Liu et al. 2015).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: XF, YL. Formal analysis: YL, LL. Funding acquisition: XF. Investigation: XF, YL, LL, JC, MG. Methodology: YL. Collection: YL, XF, LL, JC, MG. Resources: YL, LL, XF. Software: YL, XF. Supervision: XF. Validation: LL. Visualization: YL. Writing - original draft: YL. Writing - review and editing: LL, XF.

Author ORCIDs

Yixuan Li https://orcid.org/0009-0007-6862-9605
Lu Lin https://orcid.org/0000-0003-3456-3760
Xinlei Fan https://orcid.org/0000-0002-4946-4442

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Alvarez M (1976) Primer catalogo de enfermedades de plantas Mexicanas. Fitofilo 71: 1–169.

Aumentado HDR, Armand A, Phukhamsakda C, Hyde KD, Jones EG, Jayawardena RS (2024) Polyphasic identification of two novel *Colletotrichum* species causing leaf spots on mangroves in Thailand. European Journal of Plant Pathology 169(1): 1–27. https://doi.org/10.1007/s10658-024-02819-y

Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller JM, Abang MM, Zhang ZJ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39: 183–204. https://doi.org/10.1016/i.riam.2009.11.001

Cannon PF, Buddie AG, Bridge PD (2008) The typification of *Colletotrichum gloeosporioides*. Mycotaxon 104: 189–204.

Cannon PF, Damm U, Johnston PR, Weir BS (2012) *Colletotrichum*: Current status and future directions. Studies in Mycology 73: 181–213. https://doi.org/10.3114/sim0014

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051

Chen MM (2003) Forest fungi phytogeography: Forest fungi phytogeography of China, North America, and Siberia and International Quarantine of Tree Pathogens. Pacific Mushroom Research & Education Center, Sacramento, California.

Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hywel-Jones NL (2004) *Calonectria* species and their *Cylindrocladium* anamorphs, species with sphaeropedunculate vesicles. Studies in Mycology 50: 415–430. https://doi.org/10.3114/sim.55.1.213

Da Lio D, Cobo-Díaz JF, Masson C, Chalopin M, Kebe D, Giraud M, Verhaeghe A, Nodet P, Sarrocco S, Le Floch G, Baroncelli R (2018) Combined metabarcoding and multi-locus approach for genetic characterization of *Colletotrichum* species associated with common walnut (*Juglans regia*) anthracnose in France. Scientific Reports 8(1): e10765. https://doi.org/10.1038/s41598-018-29027-z

Damm U, Woudenberg JHC, Cannon PF, Crous PW (2009) *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Diversity 39: 45–87.

Damm U, Cannon PF, Woudenberg JH, Johnston PR, Weir BS, Tan YP, Shivas RG, Crous PW (2012a) The *Colletotrichum boninense* species complex. Studies in Mycology 73: 1–36. https://doi.org/10.3114/sim0002

Damm U, Cannon PF, Woudenberg JHC, Crous PW (2012b) The *Colletotrichum acutatum* species complex. Studies in Mycology 73: 37–113. https://doi.org/10.3114/sim0010

Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology 13(4): 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus (San Francisco, Calif.) 12: 13–15. https://doi.org/10.2307/2419362

Gadgil PD, Dick MA, Hood IA, Pennycook SR (2005) Fungi on Trees and Shrubs in New Zealand. Fungal Diversity Press, Department of Ecology and Biodiversity, The University of Hong Kong.

Gao YH, Sun W, Su YY, Cai L (2013) Three new species of *Phomopsis* in Gutianshan Nature Reserve in China. Mycological Progress 13(1): 111–121. https://doi.org/10.1007/s11557-013-0898-2

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61(4): 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Gorter GJMA (1977) Index of plant pathogens and the diseases they cause in cultivated plants in South Africa. Republic South Africa, Department of Agriculture Technology Services Plant Protection Research Institute, Science Bulletin.

Guerber JC, Liu B, Correll JC, Johnston PR (2003) Characterization of diversity in *Colletotrichum acutatumsensu lato* by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. Mycologia 95(5): 872–895. https://doi.org/10.1080/15572536.2004.11833047

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel HO (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–321. https://doi.org/10.1093/sysbio/syq010

Guo Z, Luo CX, Wu HJ, Peng B, Kang BS, Liu LM, Zhang M, Gu QS (2022) *Colletotrichum* species associated with anthracnose disease of watermelon (*Citrullus lanatus*) in China. Journal of Fungi 8(8): e790. https://doi.org/10.3390/jof8080790

He L, Li X, Gao Y, Li B, Mu W, Liu F (2019) Characterization and fungicide sensitivity of *Colletotrichum* spp. from different hosts in Shandong, China. Plant Disease 103(1): 34–43. https://doi.org/10.1094/PDIS-04-18-0597-RE

Jayawardena RS, Huang J, Jin B, Yan JY, Li XH, Hyde KD, Bahkali AH, Yin S, Zhang GZ (2016a) An account of *Colletotrichum* species associated with strawberry anthracnose in China based on morphology and molecular data. Mycosphere 7(8): 1147–1163. https://doi.org/10.5943/mycosphere/si/2c/6

Jayawardena RS, Hyde KD, Damm U, Cai L, Liu M, Li XH, Zhang W, Zhao WS, Yan JY (2016b) Notes on currently accepted species of *Colletotrichum*. Mycosphere 7(8): 1192–1260. https://doi.org/10.5943/mycosphere/si/2c/9

Jayawardena RS, Hyde KD, Chen YJ, Papp V, Palla B, Papp D, Bhunjun CS, Hurdeal VG, Senwanna C, Manawasinghe IS, Harischandra DL, Gautam AK, Avasthi S, Chuankid B, Goonasekara ID, Hongsanan S, Zeng X, Liyanage KK, Liu N, Karunarathna A, Hapuarachchi KK, Luangharn T, Raspe O, Brahmanage R, Doilom M, Lee HB, Mei L, Jeewon

R, Huanraluek N, Chaiwan N, Stadler M, Wang Y (2020) One stop shop IV: Taxonomic update with molecular phylogeny for important phytopathogenic genera: 76–100 (2020). Fungal Diversity 103: 87–218. https://doi.org/10.1007/s13225-020-00460-8

Juhásová G, Ivanová H, Spišák J (2005) Occurrence and spread of the parasitic microscopic fungi on walnut (*Juglans regia* L.) on various localities of Slovakia. Environmental Health Perspectives 113: 345–352. https://doi.org/10.17660/ActaHortic.2014.1050.47

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kobayashi T (2007) Index of fungi inhabiting woody plants in Japan-Host, Distribution and Literature-, Takao Kobayashi. Journal of Tree Health 11(3): 142. https://doi.org/10.11519/jjsk.50.0_57

Li FX, Chen JW, Chen Q, Liu ZY, Sun JY, Yan YT, Zhang HX, Bi Y (2023) Identification, pathogenicity, and sensitivity to fungicide of *Colletotrichum* species that causes walnut anthracnose in Beijing. Agronomy 13(1): e214. https://doi.org/10.3390/agronomy13010214

Liu XY, Jing Y, Yang JX (1995) A study on *Colletotrichum* classification of wooden plant in Shaanxi province. Journal of Northwest Forestry College 3: 29–30.

Liu F, Hyde KD, Cai L (2011) Neotypification of *Colletotrichum coccodes*, the causal agent of potato black dot disease and tomato anthracnose. Mycology 2(4): 248–254. https://doi.org/10.1080/21501203.2011.600342

Liu F, Damm U, Cai L, Crous PW (2013) Species of the *Colletotrichum gloeosporioides* complex associated with anthracnose diseases of *Proteaceae*. Fungal Diversity 61(1): 89–105. https://doi.org/10.1007/s13225-013-0249-2

Liu F, Weir BS, Damm U, Crous PW, Wang Y, Liu B, Wang M, Zhang M, Cai L (2015) Unravelling *Colletotrichum* species associated with *Camellia*: Employing ApMat and GS loci to resolve species in the *C. gloeosporioides* complex. Persoonia 35(1): 63–86. https://doi.org/10.3767/003158515X687597

Liu F, Wang M, Damm U, Crous PW, Cai L (2016) Species boundaries in plant pathogenic fungi: A *Colletotrichum* case study. BMC Evolutionary Biology 16(1): 81. https://doi.org/10.1186/s12862-016-0649-5

Liu MZ, Li CH, Cao CM, Wang LQ, Li XP, Che J, Yang HM, Zhang XW, Zhao HY, He GZ, Liu XD (2021) Walnut fruit processing equipment: Academic insights and perspectives. Food Engineering Reviews 13(4): 1–36. https://doi.org/10.1007/s12393-020-09273-6

Liu F, Ma ZY, Hou LW, Diao YZ, Wu WP, Damm U, Song S, Cai L (2022) Updating species diversity of *Colletotrichum*, with a phylogenomic overview. Studies in Mycology 101(1): 1–56. https://doi.org/10.3114/sim.2022.101.01

Luongo L, Galli M, Garaguso I, Petrucci M, Vitale S (2022) First report of *Colletotrichum fioriniae* and *C. nymphaeae* as causal agents of anthracnose on walnut in Italy. Plant Disease 106(1): e327. https://doi.org/10.1094/PDIS-05-21-1062-PDN

Ma T, Yang C, Cai F, Chen Z (2022) Morpho-cultural, physiological and molecular characterisation of *Colletotrichum nymphaeae* causing anthracnose disease of walnut in China. Microbial Pathogenesis 166: e105537. https://doi.org/10.1016/j.micpath.2022.105537

Marin-Felix Y, Groenewald JZ, Cai L, Chen Q, Marincowitz S, Barnes I, Bensch K, Braun U, Camporesi E, Damm U, de Beer ZW, Dissanayake A, Edwards J, Giraldo A, Hernández-Restrepo M, Hyde KD, Jayawardena RS, Lombard L, Luangsa-ard J, McTaggart AR, Rossman AY, Sandoval-Denis M, Shen M, Shivas RG, Tan YP, van der Linde EJ, Wingfield MJ, Wood AR, Zhang JQ, Zhang Y, Crous PW (2017) Genera of phytopathogenic fungi: GOPHY 1. Studies in Mycology 86(1): 99–216. https://doi.org/10.1016/j.simyco.2017.04.002

Mu T, Zhang Z, Liu R, Liu S, Li Z, Zhang X, Xia J (2021) Morphological and molecular phylogenetic analyses reveal three species of *Colletotrichum* in Shandong province, China. MycoKeys 85: 57–71. https://doi.org/10.3897/mycokeys.85.75944

Nie W, Yu FW, He YY (2016) Evaluation of ecological benefit of walnut planting-a case of Dayao county of Yunnan Province. Anhui Nongye Kexue 44(29): 156–160. https://www.researchgate.net/publication/331997411

Pennycook SR (1989) Plant diseases recorded in New Zealand. Plant Diseases Division, Department of Scientific and Industrial Research, Auckland, New Zealand.

Penzig AGO (1882) Fungi agrumicoli. Contribuzione allo studio dei funghi parassiti degli agrumi. Michelia 2: 385–508.

Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Prihastuti H, Cai L, Chen H, McKenzie EHC, Hyde KD (2009) Characterization of *Colletotrichum* species associated with coffee berries in Northern Thailand. Fungal Diversity 39(1): 89–109.

Qu WW, Yang KQ, Liu HX, Hou LQ (2011) Main diseases of walnut and integrated management in Shandong. Plant Protection 44: 136–140.

Rambaut A, Drummond A (2010) FigTree v. 1.3.1. Institute of Evolutionary Biology; University of Edinburgh: Edinburgh, UK.

Rayner RW (1970) A Mycological Colour Chart. Commonwealth Mycological Institute, Kew, UK.

Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA, Cannon P, Chen R, Pang J, Wang R, Zhang Y, Peng Y-Q, Sha T (2010) *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panama: Multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102(6): 1318–1338. https://doi.org/10.3852/09-244

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Savian LG, Muniz MFB, Poletto T, Maculan LG, Rabuske JE, Blume E, Sarzi JS (2019) First report of *Colletotrichum nymphaeae* causing anthracnose on *Juglans regia* fruits in Southern Brazil. Plant Disease 103(12): e3287. https://doi.org/10.1094/PDIS-06-19-1199-PDN

Sharma G, Pinnaka AK, Shenoy BD (2015) Resolving the *Colletotrichum siamense* species complex using ApMat marker. Fungal Diversity 71(1): 247–264. https://doi.org/10.1007/s13225-014-0312-7

Sharma G, Maymon M, Freeman S (2017) Epidemiology, pathology and identification of *Colletotrichum* including a novel species associated with avocado (*Persea americana*) anthracnose in Israel. Scientific Reports 7(1): 15839. https://doi.org/10.1038/s41598-017-15946-w

Shivas RG, Tan YP (2009) A taxonomic re-assessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. Fungal Diversity 39: 111–122.

Simmonds JH (1966) Host index of plant diseases in Queensland. Brisbane Queensland Department of Primary Industries Australia.

Sreenivasaprasad S, Talhinhas P (2005) Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. Molecular Plant Pathology 6(4): 361–378. https://doi.org/10.1111/j.1364-3703.2005.00291.x

Talhinhas P, Baroncelli R (2021) *Colletotrichum* species and complexes: Geographic distribution, host range and conservation status. Fungal Diversity 110(1): 109–198. https://doi.org/10.1007/s13225-021-00491-9

Talhinhas P, Baroncelli R (2023) Hosts of *Colletotrichum*. Mycosphere : Journal of Fungal Biology 14(2): 158–261. https://doi.org/10.5943/mycosphere/14/si2/4

Walker J (1980) The coelomycetes. Fungi imperfecto with pycnidia, acervuli and stromata. Australasian Plant Pathology 9(4): 120–121. https://doi.org/10.1007/BF03213663

Wang QH, Liu XH, Fan K, Duan CH, Niu SG, Wu XQ (2016) Identification and biological characteristics of pathogen from *Colletotrichum gloeosporioides*. Shandong Nongye Daxue Xuebao 47(1): 9–14.

Wang QH, Fan K, Li DW, Niu SG, Hou LQ, Wu XQ (2017) Walnut anthracnose caused by *Colletotrichum siamense* in China. Australasian Plant Pathology 46(6): 585–595. https://doi.org/10.1007/s13313-017-0525-9

Wang QH, Li DW, Duan CH, Liu XH, Niu SG, Hou LQ, Wu XQ (2018) First report of walnut anthracnose caused by *Colletotrichum fructicola* in China. Plant Disease 102(1): 247–247. https://doi.org/10.1094/PDIS-06-17-0921-PDN

Wang QH, Fan K, Li DW, Han CM, Qu YY, Qi YK, Wu XQ (2020a) Identification, virulence and fungicide sensitivity of *Colletotrichum gloeosporioides* s.s. responsible for walnut anthracnose disease in China. Plant Disease 104(5): 1358–1368. https://doi.org/10.1094/PDIS-12-19-2569-RE

Wang X, Liu X, Wang R, Lei FA, Hou X (2020b) First report of *Colletotrichum aenigma* causing walnut anthracnose in China. Plant Disease 105(1): e225. https://doi.org/10.1094/PDIS-07-20-1430-PDN

Wang XH, Liu X, Wang R, Fa L, Chen L, Xin XB, Zhang YG, Tian H, Xia MR, Hou X (2021) First report of *Colletotrichum aenigma* causing walnut anthracnose in China. Plant Disease 105(1): e225. https://doi.org/10.1094/PDIS-07-20-1430-PDN

Wang XH, Chen L, Ma QH, Liu X, Wang R, Fa L, Wang HX, Hou X, Xia MR, Yuan W (2023) First report of *Colletotrichum godetiae* causing anthracnose on walnut (*Juglans regia* and *Juglans sigillata*) in China. Plant Disease 107(8): 2544. https://doi.org/10.1094/PDIS-11-22-2625-PDN

Wei X, Yang SQ, Jiang ZG, Cui MJ, Deng JX, Zhang Y (2022) *Colletotrichum juglandis* sp. nov. (Ascomycota: Glomerellaceae) associated with walnut leaf spot in China. Phytotaxa 556(3): 256–268. https://doi.org/10.11646/phytotaxa.556.3.2

Weir BS, Johnston PR, Damm U (2012) The *Colletotrichum gloeosporioides* species complex. Studies in Mycology 73: 115–180. https://doi.org/10.3114/sim0011

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications, Academic Press, Inc., New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yang YL, Cai L, Yu ZN, Liu ZY, Hyde KD (2011) *Colletotrichum* Species on Orchidaceae in Southwest China. Cryptogamie. Mycologie 32(3): 229–253. https://doi.org/10.7872/crym.v32.iss3.2011.229

Yang HB, Cao GL, Jiang SJ, Han S, Yang CL, Wan XQ, Zhang F, Chen LH, Xiao JJ, Zhu P, Zhang DJ, He F, Xing WX (2021) Identification of the anthracnose fungus of walnut (*Juglans* spp.) and resistance evaluation through physiological responses of resistant vs. susceptible hosts. Plant Pathology 70(5): 1219–1229. https://doi.org/10.1111/ppa.13354

Yu ZF, Jiang XW, Zheng H, Zhang H, Qiao M (2022) Fourteen new species of foliar *Colletotrichum* associated with the invasive plant *Ageratina adenophora* and surrounding crops. Journal of Fungi 8(2): e185. https://doi.org/10.3390/jof8020185

Zapata M, Rodríguez-Serrano E, Castro JF, Santelices C, Carrasco-Fernández J, Damm U, Palfner G (2024) Novel species and records of *Colletotrichum* associated with native woody plants in south-central Chile. Mycological Progress 23(1): 18. https://doi.org/10.1007/s11557-024-01956-2

Zhang Q, Nizamani MM, Feng Y, Yang YQ, Jayawardena RS, Hyde KD, Wang Y, Li C (2022) Genome-scale and multi-gene phylogenetic analyses of *Colletotrichum* spp. host preference and associated with medicinal plants. Mycosphere 14(2): 1–106. https://doi.org/10.5943/mycosphere/14/si2/1

Zhang L, Yin YQ, Zhao LL, Xie YQ, Han J, Zhang Y (2023a) Two new species of *Colletotrichum* (Glomerellaceae, Glomerellales) causing walnut anthracnose in Beijing. MycoKeys 99: 131–152. https://doi.org/10.3897/mycokeys.99.106812

Zhang YX, Chen JW, Manawasinghe IS, Lin YH, Jayawardena RS, McKenzie EHC, Hyde KD, Xiang MM (2023b) Identification and characterization of *Colletotrichum* species associated with ornamental plants in Southern China. Mycosphere 14(2): 262–302. https://doi.org/10.5943/mycosphere/14/si2/5

Zhu YF, Yin YF, Qu WW, Yang KQ (2014) Occurrence and spread of the pathogens on walnut (*Juglans regia*) in Shandong Province, China. Acta Horticulturae 1050(1050): 347–351. https://doi.org/10.17660/ActaHortic.2014.1050.47

Zhu YZ, Liao WJ, Zou DX, Wu YJ, Zhou Y (2015) First report of leaf spot disease on walnut caused by *Colletotrichum fioriniae* in China. Plant Disease 99(2): 289–289. https://doi.org/10.1094/PDIS-09-14-0938-PDN

Supplementary material 1

Strains of the *Colletotrichum* species with details of host, location and GenBank accessions of the sequences

Authors: Yixuan Li, Lu Lin, Jing Cao, Mingxu Gan, Xinlei Fan

Data type: docx

Link: https://doi.org/10.3897/mycokeys.108.125382.suppl1

Supplementary material 2

The statistics of ML trees in this study

Authors: Yixuan Li, Lu Lin, Jing Cao, Mingxu Gan, Xinlei Fan

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